Biophysical Chemistry 241 (2018) 8–14

Contents lists available at ScienceDirect

Biophysical Chemistry

journal homepage: www.elsevier.com/locate/biophyschem

Research Article

Carboplatin as an alternative to Cisplatin in chemotherapies: New insights at single molecule level

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ARTICLE INFO

Keywords:
Carboplatin
Cisplatin
Single molecule force spectroscopy
DNA cancer chemotherapy

ABSTRACT

Here we report a new study performed at single molecule level on the interaction of the antineoplastic drug Carboplatin and the DNA molecule - the main target of the drug inside cells in cancer chemotherapies. By using optical tweezers, we measure how the mechanical properties of the DNA-Carboplatin complexes changes as a function of the drug concentration in the sample, for two different ionic strengths ([Na] = 150 mM and [Na] = 1 mM). From these measurements, the binding mechanism and the physicochemical (binding) parameters of the interaction were inferred and directly compared to those obtained for the precursor drug Cisplatin under equivalent conditions. As the main conclusion, we show that Carboplatin binds preferentially forming covalent monoadducts in contrast to Cisplatin, which is hydrolyzed easier and presents a higher efficiency in forming covalent diadducts along the double-helix. In addition, we explicitly show that Carboplatin is much less sensitive to ionic strength changes when compared to Cisplatin. These findings provide new insights on the interactions of platinum-based compounds with the DNA molecule, being important to improve the current treatments and in the development of new antineoplastic agents.

1. Introduction

Since the end of the 1970s, Platinum-derived drugs have been used in chemotherapeutic treatments against cancers of testicle, ovary, esophagus, lung, head, neck, and other organs. The first generation of these drugs started with Cisplatin (*cis-diammine-dichloroplatinum (II)*), which is still the compound most commonly used in chemotherapeutic treatments. Nevertheless, its use has been accompanied by a number of undesirable side effects (*e.g.*, nephrotoxicity and ototoxicity), and by the development of drug resistance. Based on these limitations, new Platinum complexes have been developed, such as Carboplatin (*cis-diammine (cyclobutane-1,1-dicarboxylate-O, O') platinum (II)* [1], which is a second generation platinum-based compound that exhibits an antineoplastic activity similar to Cisplatin, also presenting a similar mechanism for adduct formation upon binding to the double-helix [2]. Carboplatin also exhibits a preference for binding to guanine N7 and adenine N7, however, there are reports of a considerable affinity for cytosine, as well as conformational changes between A- and B-DNA [2].

Carboplatin has been used specifically in many cancer treatments, such as ovary [3–5], lung [6], and some specific types of head tumors [7], despite being less efficient than Cisplatin in certain specific cases [8–12]. There is an increasing interest of the scientific community in investigating the molecular mechanism of Carboplatin action [13–16], in particularly in the study of its interaction with the DNA molecule, in order to guide the development of more efficient compounds. Fig. 1, shows the chemical structure of the Carboplatin molecule, which is a divalent cationic drug when fully hydrolyzed in solution, becoming identical to the active (hydrolyzed) form of Cisplatin, also shown in the figure for comparison purposes.

Some studies have already investigate the DNA-Carboplatin interaction [17–23], but none have investigated the complexes formation at single molecule level, nor have studied the role of the ionic strength of the surrounding buffer solution on the formation of such complexes. Here we address these important points by using single molecule force spectroscopy to measure the mechanical properties of the DNA-Carboplatin complexes as a function of the drug concentration for different ionic strengths. Based on the results obtained, the physicochemical (binding) parameters of the DNA-Carboplatin interaction were determined [24, 25] for different ionic strengths, giving new insights and useful quantitative information about such system. Finally, the results obtained were compared to those found for the precursor compound Cisplatin, giving new insights on the usage of Carboplatin as a substitute of Cisplatin in cancer chemotherapies.

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https://doi.org/10.1016/j.bpc.2018.07.004
Received 3 July 2018; Accepted 20 July 2018
Available online 25 July 2018
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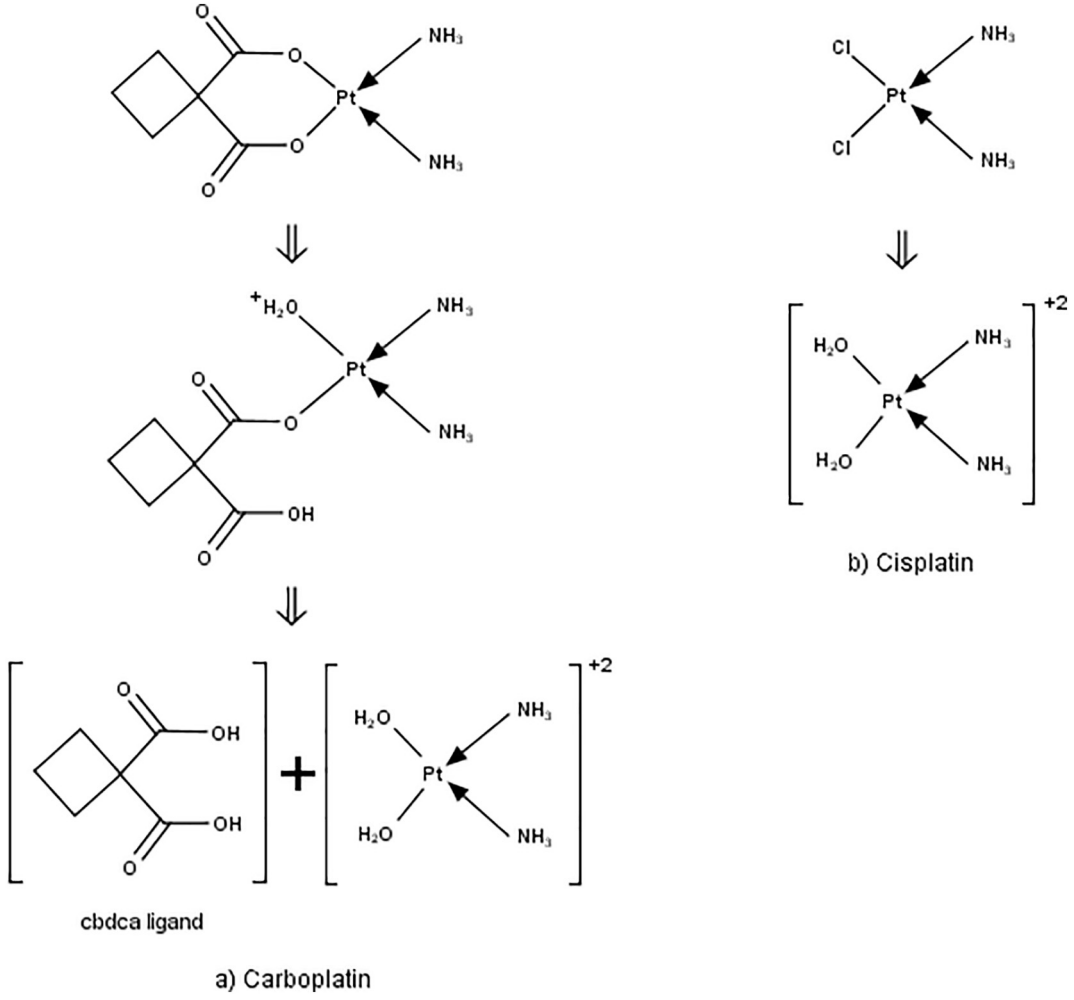

Fig. 1. (a) Chemical structure and hydrolization of the Carboplatin molecule. The fully hydrolized form is achieved when the 1,1-cyclobutanedicarboxylate (cbdca) ligand leave the platinum core, being substituted by two water molecules. (b) Chemical structure and hydrolization of the Cisplatin molecule shown here for comparison purposes. The active form is achieved when the two chlorides are substituted by water molecules.

2. Materials and methods

2.1. Sample preparation

The samples contain biotin-labeled λ-DNA molecules (New England Biolabs) in a Phosphate Buffer Saline (PBS) solution. We attach one end of the DNA molecules to a microscope coverslip surface previously coated with streptavidin, while the other end is attached to a polystyrene microsphere of 3 μm in diameter, also coated with streptavidin (Bangs Labs). Our measurements were made for two different ionic strengths, using distinct PBS solutions, as detailed in Table 1. More details about the experimental methods and procedures can be found in a previous publication [26].

2.2. Experimental procedure

The experimental setup used in this work consists of a 1064 nm solid state laser (Altechna) operating in TEM_{00} mode, mounted on a Nikon Ti−U inverted microscope with a 100× NA 1.4 objective. This tweezers was previously calibrated using the Stokes method to determine the trap stiffness. The equipment is then used to trap a single polystyrene microsphere attached to a DNA molecule. The stretching of the biopolymer is performed by moving the stage of the microscope with the assistance of a piezoelectric motor (Newport Corp.). Simultaneously we monitor the changes on the position of the microsphere inside the optical potential well using videomicroscopy.

Table 1
Composition of the different PBS buffers used.

Buffer	NaCl	Na_2HPO_4	NaH_2PO_4
PBS [Na] = 150 mM	140 mM	4.375 mM	1.25 mM
PBS [Na] = 1 mM	0	0.4375 mM	0.125 mM

To work in the entropic regime, we imposed to the DNA-Carboplatin complexes an applied maximum force of 5 pN, in order to not disturb the chemical equilibrium by mechanical manipulation. The Marko-Siggia worm-like chain (WLC) [27] equation was used to fit the experimental data and extract the two relevant mechanical parameters: the contour length L and the persistence length A. In addition, we have also used high forces (tens of pN) to investigate the role of Carboplatin on the secondary structure of the double-helix, and in particular on the DNA denaturation plateau usually obtained at ~ 65 pN.

Before adding Carboplatin to the sample, a bare DNA molecule is chosen and characterized in a series of stretching measurements, performed repeatedly to guarantee that its average mechanical properties are close to those expected for the λ-DNA: $A_0 \sim 45$ nm and $L_0 \sim 16.5$ μm. Then we carefully introduce Carboplatin into the sample chamber using micropipettes, keeping the same DNA molecule tethered. We wait ~ 0.5 h for the DNA-Carboplatin complexes to equilibrate, then we start the next stretching measurements. We determine

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